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13. SUPPLEMENTARY NOTES

14. ABSTRACT:

The focus of these experiments was to test the hypothesis that dermal application of military jet fuel (JP-8) is immune suppressive. Three specific aims were designed to test this hypothesis: 1) Does dermal exposure to JP-8 induce immune suppression? 2) What are the mechanisms involved? 3) Can you reverse the immune suppressive effects of JP-8? Applying JP-8 to the skins of mice does suppress the immune response. The mechanisms underlying immune suppression appear to involve the production of immune regulatory cytokines and biological response modifiers by JP-8-treated keratinocytes. In particular we found that the immune suppression induced by JP-8 was mediated by Prostaglandin E_2 and interleukin-10. Understanding the mechanism(s) involved provided a method to reverse immune suppression. Neutralizing the activity of Prostaglandin E_2 and interleukin-10 *in vivo* blocked JP-8-induced immune suppression. Prostaglandin E_2 production *in vivo* was blocked with a selective cyclooxygenase-2 inhibitor. Because this class of drugs is now available for use in humans(i.e., Celebrex), the data generated here suggest that using selective cyclooxygenase-2 inhibitors to block prostaglandin-2 production *in vivo* may present the best way to prevent JP-8-induced immune suppression in USAF personnel.

15. SUBJECT TERMS

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APPENDIX

Manuscripts published:

Ullrich, S.E. Dermal application of JP-8 jet fuel induces immune suppression. *Toxicological Sciences* **52**:61-67, 1999.
Ullrich, S.E. & Lyons, H.L. Mechanism involved in the immunotoxicity induced by dermal application of JP-8 jet fuel. *Toxicological Sciences* **58**:290-298, 2000.
Ramos, G., Nghiem, D.X., Walterscheid, J. P., & Ullrich, S.E. Dermal application of jet fuel suppresses secondary immune reactions. *Toxicology and Applied Pharmacology* **180**:136-144, 2002.

Scientific Personnel:
Stephen E. Ullrich, Ph.D. (Professor; Principal Investigator)
Heather Lyons, BS (Research Assistant I)
Capt. Jerry Ramos, USAF (Graduate Assistant I)
Jeffrey Walterscheid, BS (Graduate Assistant I)
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Inventions/Patents/Discoveries:
None

Collaborators/Consultants:

1. Drs. Mahendra Kabbur and James McDougal, Operational Toxicology Branch, Wright-Patterson Air Force Base. We measured IL-10 levels in extracts taken from the skin of JP-8-treated rats.

2. Dr. Frank Witzmann, Indiana University, Columbus IN. Provide samples of dermal skin cells treated with JP-8 for proteomic analysis.

3. Dr. Geraldine Grant, George Mason University, Fairfax, VA. Provide RNA from dermal cells treated with JP-8 for gene chip analysis.

4. Dr. Vijayalaxmi, The University of Texas, Health Science Center At San Antonio. Provide samples of bone marrow and blood from JP-8-treated mice for micronuclei analysis.

Honors/Awards:
None

Key Findings/Results Accomplishments:

During the project period we completed experiments designed to answer all three of our original research questions (1-3). We also addressed two additional questions. First we determined that JP-8 exposure suppresses secondary immune reactions (4). Moreover, we discovered that the base kerosene fuels, commercial grade Jet-A is also immune suppressive. This suggests that the immune suppression observed with JP-8 is a function of the base fuel and does not results form the antifreeze, anti-corrosive reagent and the anti-static agent that the USAF adds to Jet-A to make it JP-8.

1) Does dermal application of JP-8 induce immune suppression? The answer is yes, applying JP-8 to the skin of mice suppresses the immune reaction. However, JP-8 is not a pan-immunosuppressive agent. Although cellular immune reactions such as delayed type hypersensitivity and T cell proliferation were sensitive to the immune suppressive effects of JP-8; antibody formation was not affected. This demonstrates that JP-8 has a selective effect on immune function. It may suggest that immune functions that depend on cell mediated immunity, such as clearance of intracellular pathogens and virus may be more susceptible to the suppressive effects of JP-8 than antibody mediated immune function. The immune suppression induced by dermal application of JP-8 is dose dependent. We found a threshold level of 250 to 300 µl of un-diluted JP-8 ($\approx$240 µg) to the skin was required to induce immune suppression. This dose could be applied in one large dose or fractionated over time. After a single dermal exposure the immune suppression persisted for three weeks, after which time the immune function of the JP-8-treated mice returned to normal.

2) What are the mechanisms involved? We demonstrated that JP-8 induces systemic immune suppression via the release of immune modulatory biological response modifiers and cytokines. In particular we found that the immune suppression induced by JP-8 was mediated by prostaglandin E_2 and interleukin-10. We propose that JP-8 activates keratinocytes to secrete prostaglandin E_2, which induces a cascade of events leading to serum interleukin-10 secretion and immune suppression.

3) Can you reverse the immune suppressive effects of JP-8? Yes, by targeting cytokine production *in vivo*. We found that we can reverse the immunosuppressive effects of JP-8 by injecting mice with antibodies to IL-10, reversing the effects of IL-10 by injecting the immune adjuvant IL-12 or by preventing the suppressive cascade from starting in the first place by suppressing prostaglandin E_2 synthesis. One of the best ways to do this is with a selective cyclooxygenase-2 inhibitor. By injecting JP-8-treated mice with the active form of Celebrex, SC 236, we found total reversal of JP-8-induced immune suppression. Because this drug is

now available and used to treat chronic disease such as arthritis, with minimal side effects, we suggest this is the best way to overcome JP-8-indued immune suppression in exposed USAF personnel.

4) Does JP-8 exposure suppress secondary Immune reactions? During the past year we directed our attention to the effects of dermal JP-8 exposure on immunological memory. The most dramatic improvement in public health during the past century came as a result of vaccination to combat infectious disease. If dermal JP-8 exposure can suppress immune memory, it may suggest decreased protection against microbial infection. We tested this hypothesis in our mouse model. We found that JP-8 exposure suppresses immunological memory, in a dose-dependent manner. In addition, like previous studies, suppression of the memory reaction occurred regardless of whether the JP-8 was applied one time or the dose was fractionated over several days. Moreover, we found that injecting JP-8-treated mice with SC 236, the selective cyclooxygenase inhibitor, blocked JP-8-induced suppression of immunological memory.

5) Does dermal exposure to commercial jet fuel (Jet-A) suppress the immune response? We also carried out identical experiments with commercial grade JetA, the base kerosene fuel for JP-8. We found that dermal application of JetA also suppressed immunological memory. The mechanism appears to be identical in that SC 236 blocked immune suppression induced by Jet A. This suggests that the immunosuppressive properties of JP-8 reside within the base kerosene fuel and is not a function of the military specific additives.

Transitions/Technology Transfers:
None